Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US05/001755

International filing date: 20 January 2005 (20.01.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/616,390
Filing date: 06 October 2004 (06.10.2004)

Date of receipt at the International Bureau: 03 March 2005 (03.03.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

1287443

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

February 22, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/616,390*
FILING DATE: *October 06, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US05/01755*

Certified by

Jon W. Dudas

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

100604 20427 U.S. PTO

Please type a plus sign (+) inside this box→ +

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c)

19249 60/616390 U.S. PTO 100604

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City & State or Foreign Country)
Stephen E.	BARRY	Alamo, CA

__ *Additional inventors are being named on the ___ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (200 characters max)

Nanoparticulate Formulations of Magnetic Material and Bioactive Agents

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

X Customer Number 23979 → Bar Code Label: 23979 PATENT TRADEMARK OFFICE

OR __ *Firm or Individual Name:* _____

Address: _____

City: _____ State: _____ ZIP: _____

Country: _____ Phone: _____ Fax: _____

ENCLOSED APPLICATION PARTS *(check all that apply)*

X Specification – *Number of Pages* 18 __ CD(s) – *Number* _____

__ Drawing(s) – *Number of Sheets* _____ X Other (specify): Return postcard

__ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION

X Applicant claims small entity status. See 37 CFR 1.27.
__ A check or money order is enclosed to cover the filing fees.
__ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Acct. No.: _____
X Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($)

$ 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

X No
__ Yes, the name of the U.S. Government agency & the contract number are: _____

Respectfully submitted,

SIGNATURE: Jacqueline S Larson

Date: 10/06/2004

REGISTRATION NO. 30,279

TYPED NAME: Jacqueline S. Larson

TELEPHONE: (408) 615-0502

Docket Number: ALNS-037Pr ~~036Pr~~

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

Express Mail Label No.: ER210423004US

Date: October 6, 2004

Express Mail Label No.: ER210423004US Date of Deposit: October 6, 2004

Nanoparticulate Formulations of Magnetic Material and Bioactive Agents

FIELD OF THE INVENTION

The present invention is directed to the field of delivery of bioactive agents via hydrogel nanoarticles comprising magnetic material, and the use thereof in therapeutic treatments.

BACKGROUND OF THE INVENTION

Chemotherapeutics are widely used in the treatment of cancer. While somewhat efficacious, the toxicity of the chemotherapeutics is severe and harmful, remission is often incomplete, and the eventual regrowth and spread of cancerous tissue is the norm.

Platinum chemotherapeutic use is increasing due in part to efficacy in a range of different cancers and demonstrated utility in new and efficacious chemotherapeutic cocktails. Cisplatin was the first platinum-based chemotherapeutic commercialized. To reduce undersirable toxicity in healthy cells and tissues, cisplatin structure has been modified by attaching various anionic and cationic ligands to affect solubility, toxicity, tissue and cellular distribution, and metabolism. For example, carboplatin is a cisplatin derivative that replaces the two chloride ligands of cisplatin with a low molecular weight dicarboxylate ligand.

Platins have also been covalently conjugated to water-soluble, synthetic polymers, such as N-(2-hydroxypropyl)methacrylamide (HPMA) (Burtles et al., Hum. Exp. Toxicol., 1998, 17, 93-104); natural polymers, such as dextran; and proteins, such as albumin (Schutte M.T., et al., Crit. Rev. Ther. Drug Carrier Syst., 1999, 16, 245-288); for purposes such as altering biodistribution, reducing toxicity, reducing multi drug resistance (MDR) efflux, and prolonging activity. Size and solubility provide a basis for

understanding the differences between the drug compared to the drug conjugate. Attachment to hydrophilic polymers produces larger structures with decreased diffusivity and increased water and blood solubility. Nonspecific membrane penetration is thus substantially reduced. Such drug-polymer conjugates thus reside in the bloodstream longer in comparison to the drug alone and may therefore be passively targeted to tissues, such as solid tumors, that have a leaky vasculature.

Efforts at localizing chemotherapeutics to the cancerous tissues, for instance through the attachment of chemotherapeutics to monoclonal antibodies which bind to receptors over-expressed on cancer cells, has thus far been a modest success at best.

Hyperthermia, in which the temperature of cancerous tissue is raised, has demonstrated some efficacy in treating multiple types of cancer. One way that the temperature can be raised is through first localizing magnetic articles within a tumor, and subsequently heating the magnetic articles by subjecting them to an alternating magnetic field. Magnetic articles used for this type of therapeutic regimen may be made using several methods; one such method is described by Tan, et.al., in PCT application WO 01/88540 A1. It has been reported that certain magnetic particles can reach temperatures in excess of 150 °C. However, to effectively heat the tumor mass, particles require a high level of accumulation that is very difficult to achieve.

Thus, although the above cancer treatment methods are somewhat efficacious, improved cancer treatments are urgently needed.

SUMMARY OF THE INVENTION

This invention is directed to discrete articles, preferably nanoarticles, comprising i) a core comprised of superparamagnetic material of less than 100 nm in diameter, ii) a scaffold encapsulating the superparamagnetic material comprised of organic polymers, and iii) bioactive agents comprised of platinum that are attached to the scaffold via one or more coordination bonds.

The magnetic hydrogel nanoarticles of the invention are preferably from about 5 nm to about 100 nm in diameter. The size of the nanoarticles allows their use as bioactive entities in mammals. To avoid uptake by the reticuloendothelial system,

nanoarticles are preferably less than 100 nm. To avoid renal clearance, nanoarticles are preferably larger than 5 nm.

Preferred superparamagnetic cores are comprised of iron oxides, such as magnetite, maghemite, and greigite.

Preferred encapsulating polymers are comprised of multiple carboxylate moieties ("polycarboxylates"). The carboxylate moieties of this polymer can serve at least two purposes, first to bind to the iron oxide core and secondly to form coordination bonds with platinum. Polycarboxylate materials that may be used in the present invention include carbohydrates with each chain comprised of multiple carboxylate moieties, as well as acrylic polymers with carboxylate side chains such as polyacrylate, polymethacrylate. Preferred copolymers for use in the present invention are polyethylene glycol – polycarboxylate copolymers, more preferably polyethylene glycol – polycarboxylate block copolymers, and most preferably polyethylene glycol – polymethacrylate (acid or salt form) or polyethylene glycol – polyacrylate (acid or salt form).

Polyethylene glycol can provide stealth behavior *in vivo,* as is well-described in the literature, and also serve as a tether for attaching targeting ligands to the nanoarticles. For the PEG chain, a preferred molecular weight is in the range of 2000 to 10,000 Daltons. For the polyacrylate, a preferred molecular weight is in the range of 2000 to 6000 Daltons.

The articles of the invention may further comprise recognition elements ("REs") that bind to certain biomolecules found in pathogenic tissue, such as certain cellular receptors that are overexpressed on the surface of some cancer cells. The number of REs per nanoarticle can range from 2 to about 1000, preferably from 2 to 500. The nanoarticles may optionally further comprise more than one type of RE. As used herein, a RE "type" is defined as an RE of a specific molecular structure. An additional advantage of the present invention is that multiple RE types with complementary features may be incorporated into a single nanoarticle.

Under the influence of an alternating magnetic field, the temperature of the magnetic material and thus of the article may be raised through various mechanisms described in the literature. This heating results in the disruption of the bonds holding

the article of the instant invention together and/or the coordination bonds retaining the bioactive platinum agent within the complex, thus causing the accelerated release of the bioactive agent from the article. Preferably, some individual platinum atoms are each connected to two or more distinct polymer chains, which contributes to the stabilization of the nanoarticle network.

In the case of chemotherapeutics, elevated temperature is known to increase the toxicity and anti-cancer potency of chemotherapeutics, so the localized heating generated by the magnetic material is expected to increase the potency of the released chemotherapeutic. While the heating itself is expected to result in the death of a portion of cancer cells in a tumor, heating of the entire tumor may not be achieved. However, the heating of the surrounding tissue is expected to aid in the diffusion of the chemotherapeutic into the tumor, resulting in the killing of more cancer cells. Outside of the applied magnetic field wherein the articles are heated, the articles of the invention will release the chemotherapeutic toxins only to a limited degree, such that the toxicity of the chemotherapeutic both systemically and in certain organs, such as in the heart, liver, kidney, and lung, is much reduced.

At least three targeting mechanisms localize the magnetic hydrogel nanoarticles of the invention at the tumor site. First, the article size will be designed such that preferential tumor accumulation occurs through the well-documented enhanced permeability and retention (EPR) effect. Second, targeting (i.e., recognition) elements on the surface of the nanoartcles will help localize the articles to the tumor site by binding tumor-associated antigens. Several different targeting agents or REs may be utilized, including specific small molecule or peptide ligands, as well as antibodies or antibody fragments (*e.g.*, scFv). Finally, application of a localized alternating magnetic field (AMF) at the tumor site can be used to cause localized release of the chemotherapeutic. The AMF will also lead to heating within the cancer tissue, killing some tumor cells by this mechanism and, perhaps more importantly, increasing the vulnerability of the tumor cells to the released cytotoxic payload.

Advantageously, the location of the articles of the instant invention within the mammalian body may be determined using magnetic resonance imaging (MRI) of the superparamagnetic cores.

The invention is further directed to methods of synthesizing these nanoarticles.

DETAILED DESCRIPTION OF THE INVENTION

The terms "a" and "an" mean "one or more" when used herein.

As used herein, the terms "nanoarticle scaffold", "hydrogel scaffold" and "scaffold" are used interchangeably and refer to the portion of the nanoarticle (the polymeric matrix structure) that incorporates the magnetic material.

This invention is directed to discrete articles, preferably nanoarticles, comprising i) a core comprised of superparamagnetic material of less than 100 nm in diameter, ii) a scaffold encapsulating the superparamagnetic material comprised of organic polymers, and iii) bioactive agents comprised of platinum that are attached to the scaffold via one or more coordination bonds.

The nanoarticles of the invention are preferably from about 5 nm to about 100 nm in diameter. The size of the nanoarticles allows their use as bioactive entities in mammals. To avoid uptake by the reticuloendothelial system, nano-articles are preferably less than 100 nm. To avoid renal clearance, nanoarticles are preferably larger than 5 nm.

Preferred superparamagnetic cores are comprised of iron oxides, including maghemite and magnetite. The iron oxide cores may be fabricated or may be purchased, for instance from companies such as Micromod Partikeltechnologie, GmbH (Rostock, Germany). Compared to the superparamagnetic particles which can be purchased, superparamagnetic particles formed as discussed below can facilitate incorporation of chemotherapeutics and recognition elements.

Superparamagnetic materials useful in the present invention can be formed through several methods. For instance, Fe(II) is prepared by dissolving $FeSO_4 7H_2O$ in water, and an Fe(III) solution is prepared by dissolving $FeCl_3 6H_2O$. These aqueous phases can then be combined in a reverse microemulsion. Addition of a base results in the formation of a magnetite colloid. This colloid can then be incorporated into a hydrogel as described below.

The iron oxide colloids may also be formed following the procedure published by Hyeon, et al. (*J Am Chem Soc* (2001) **123** 12798-12801). Compared to other methods,

this method allows careful control of the iron oxide surface chemistry. This method results in oleic acid coupled to the surface of the iron oxide with a carboxylate moiety forming coordination bonds with iron atoms on the surface of the iron oxide core. The hydrocarbon chain of the oleic acid provides aqueous solubility.

As referred to herein, "platinum", typically used in the context of a platinum complex or compounds, refers to a platinum metal atom bound to one or more ligands. The platinum atom may carry a formal charge, such as in the case of platinum salts such as K_2PtCl_4, potassium tetrachloroplatinate, in which the platinum carries a formal charge of (-2), or may carry no formal charge, as in cisplatin, $PtCl_2(NH_3)_2$. The platinum metal atom may exist in various oxidation states, such as Pt(0), Pt(II), or Pt(IV). The platinum species can be in any coordination state, but is typically four-coordinate.

Presently preferred platinum chemotherapeutic agents are in the IInd or IVth oxidation state. In one preferred embodiment, platinum (II) compounds are incorporated into the article through carboxylate coordination bonds. Preferred platinum (II) compounds are of the general formula cis-$[PtX_2(NHR_1R_2)(NHR_3R_4)]$ where X is an anion such as chloride, nitrate, or nitrite ions where one or both of the anions coordinated with a particular platinum atom are displaced by carboxylate ligands in the process of incorporation into the nanoarticles of the invention. Each of R_1, R_2, R_3, and R_4 is independently selected from the group consisting of hydrogen, lower alkyl unsubstituted or substituted with a halo group or an alkyl group, lower alkenyl unsubstituted or substituted with a halo group or an alkyl group, lower cycloalkyl unsubstituted or substituted with a halo group or an alkyl group, and lower cycloalkenyl unsubstituted or substituted with a halo group or an alkyl group, or R_1 and R_2 together form an alkyl or an alkenyl bridge, or R_3 and R_4 together form an alkyl or alkenyl bridge.

In another embodiment of the invention, multinucleate platinum agents are incorporated into the nanoarticles. Such agents may form more stable networks by forming three or more coordination bonds with polyanion components.

Preferred encapsulating polymers are comprised of multiple carboxylate moieties ("polycarboxylates"). The carboxylate moieties of this polymer can serve at least two purposes, first to bind to the iron oxide core and secondly to form coordination bonds with platin structures. Polycarboxylate materials that may be used in the instant

invention include carbohydrates with each chain comprised of multiple carboxylate moieties, as well as acrylic polymers with carboxylate side chains such as polyacrylate, polymethacrylate. Preferred copolymers for use in the instant invention are polyethylene glycol (PEG) – polycarboxylates, more preferably polyethylene glycol – polycarboxylates, and most preferably polyethylene glycol – polymethacrylate (sodium salt), or polyethylene glycol – polyacrylate (sodium salt). Polyethylene glycol can provide stealth behavior in vivo as is well-described in the literature, and also serve as a tether for attaching targeting ligands to the nanoparticles. For the PEG chain, a preferred molecular weight is in the range of 2000 to 10,000 Daltons. For the polyacrylate block, a preferred molecular weight is in the range of 2000 to 6000 Daltons.

Preferred carbohydrates for initial investigation are inulin derivatives, hyaluronic acid, and colominic acid (pectins and alginates, both multi-acid carbohydrates that have been used for various surgical/device/delivery purposes, are preferably avoided due to their high viscosity and lack of information on their use in circulating materials, although future information may prove them to be suitable for use in the present invention). Additional carbohydrates, particularly those with targeting or anti-cancer properties unto themselves, may be employed in the future.

Inulin, consisting mainly of linear β–1,2 linked polyfructose with a glucopyranose unit at the reducing end, has been used extensively as an i.v. injection to assess kidney function. It can be readily modified with carboxylate groups through the use of cyclic anhydrides such as succinic anhydride, aconitic anhydride. Presently preferred functionalization levels include one acid group per saccharide repeat. Inulin with ten repeats (that is, Degree of Polymerization (DP) = 10) to DP = 70 or higher may be used.

Polysialic acid (α-2,8 linkage) is very water soluble, behaving similarly to inulin. Polysialic acid is commercially available at a MW of 10,000 Da, which is a good size for constructing the nanoarticles of the instant invention. Advantages suggesting use are its natural occurrence in humans and its use as "nature's stealth" by bacteria.

Hyaluronic acid, a β-1,4 linked D-glucuronic acid and β-1,3 linked N-acetyl-D-glucosamine polysaccharide, is used as an injectable into joints to ease osteoarthritis. The receptor CD-44 binds to hyaluronic acid, overexpressed by certain cancer cells. Thus, the inclusion of hyaluronic acid may help localize the articles in these cancers.

Other carbohydrates or carboxylate-derivatized carbohydrates may also be used in the instant invention. For example, the carbohydrate region may be derived from simple sugars, such as N-acetylglucosamine, N-acetylgalctosamine, N-acetylneuraminic acid, neuraminic acid, galacturonic acid, glucuronic acid, ioduronic acid, glucose, ribose, arabinose, xylose, lyxose, allose, altrose, apiose, mannose, gulose, idose, galactose, fucose, fructose, fructofuranose, rhamnose, arabinofuranose, and talose; a disaccharide, such as maltose, sucrose, lactose, or trehalose; a trisaccharide; a polysaccharide, such as cellulose, starch, glycogen, alginates, inulin, pullulan, dextran, dextran sulfate, chitosan, glycosaminoglycans, heparin, heparin sulfate, hyaluronates, tragacanth gums, xanthan, other carboxylic acid-containing carbohydrates, uronic acid-containing carbohydrates, lactulose, arabinogalactan, and their derivatives, and mixtures of any of these; or modified polysaccharides. Other representative carbohydrates include sorbitan, sorbitol, chitosan and glucosamine.

The carboxyl, amine and hydroxyl groups of the carbohydrates can be modified, or replaced, to include crosslinking groups, other functionalities, or combinations thereof. For example, hydroxyl moieties may be used as attachment points for incorporation of carboxylate moieties on carbohydrate chains via the addition of succinic or malic anhydride, preferably in an aprotic solvent with base present. Additional coupling technologies known in the art of bioorganic chemistry (see, for example, G. Hermanson, *Bioconjugation Techniques*, Academic Press, San Diego, 1996, pp 27-40,155, 183-185, 615-617; and S. Hanesian, *Preparative Carbohydrate Chemistry*, Marcel Dekker, New York, 1997) may be used to modify carbohydrate structures to make the structures more amenable for use in the instant invention.

Carbohydrate-based building blocks may also be prepared by chemoenzymatic methods (Martin, B. D. et. al., *Macromolecules*, 1992, 25, 7081), for example in which *Pseudomonas cepacia* catalyzes the transesterification of monosaccharides with vinyl acrylate in pyridine or by the direct addition of an acrylate (Piletsky, S., Andersson, H., Nicholls, *Macromolecules*, 1999, 32, 633-636). Other functional groups may be present, as numerous derivatized carbohydrates are known to those familiar with the art of carbohydrate chemistry.

In addition to network formation through coordination bonds, multi-acid chlorides (readily commercially available) or certain multi-acid anhydrides (for instance, diacetic acid anhydrides) may be used to crosslink carbohydrate components. Such ester bond formation is expected to stabilize the network structure. Degradable linkages can also be included through the use of polylactide, polyglycolide, poly(lactide-co-glycolide), polyphosphazine, polyposphate, polycarbonate, polyamino acid, polyanhydride, and polyorthoester – based building blocks, among others. Additionally, degradable linkages may be used to attach polymerizable moieties to carbohydrates. For instance, inulin-multi succinate contains ester moieties that connect the inulin carbohydrate backbone to platinum atoms used to generate the scaffold of the present invention.

The nanoarticle scaffolds and the scaffold breakdown products of this invention are designed to be non-toxic and eliminated from the body.

The magnetic hydrogel nanoarticles of the invention may be further comprised of targeting agents or recognition elements (REs) that bind to certain biomolecules found in cancerous tissue, such as certain cellular receptors that are overexpressed on the surface of cancer cells. Unless otherwise specifically indicated, the terms "targeting agent" and "recognition element" are used interchangeably herein.

Tumor-associated targets include folate receptors, transferrin receptors, erbB1, erbB2, erbB3, erbB4, CMET, CEA, EphA2, carcinoembryonic (CEA) antigen, mucin antigens, including Muc-1, cellular adhesion proteins, and the cluster differentiation (CD) antigen family, including CD-9, CD-20, CD-30, CD-33, CD-40, CD-44, CD-53, CD-56, CD-70, and CD-71. Vascular targets associated with multiple pathologies, including cancer, include VEGFR-1, VEGFR-2, integrins (including integrin $\alpha v\beta 3$ and integrin $\alpha v\beta 5$), and aminopeptidase-N (also denoted as CD-13). Additional targets are extracellular proteins such as matrix metalloproteinases (MMPs), the collagen family, and fibrin.

The REs can be linked either directly or through a linker molecule to the surface of the nanoarticle. In a linker configuration, part or all of the REs are "displayed" at the end terminus of the tether. Therefore, in one application of the invention, the articles consist of REs displayed on a hydrogel scaffold. In another embodiment of the invention, the articles consist of an RE, such as a high affinity peptide, linked to the

surface of the article core scaffold via a linker molecule, the linker comprising preferentially polyethylene glycol (PEG). The PEG linker can be linear with reactive functionalities at both of the chain terminals. The PEG linker can also be multi armed, for instance possessing three, four, five, six, eight arms or more, with two or more of the arms possessing reactive functionalities that can be used to attach the PEG to the nanoarticle scaffold and the RE to the PEG.

For each of these embodiments, it is possible to functionalize the articles with several coupling strategies, varying both the order of addition of the different components and the reactive chemical moieties used for the coupling.

The components may be attached to one another in the following sequences. The polymer scaffold is first reacted with a di-functional PEG-containing tether, followed by functionalization of the free terminus of a portion of the PEG chain with a RE. Alternatively, the RE is coupled first to the PEG-containing tether, followed by the attachment of the other PEG terminus to the scaffold.

Several combinations of reactive moieties can be chosen to attach the RE to the tether and to attach the tether to the nanoarticle scaffold. In using a series of orthogonal reaction sets, varying some of the scaffold building blocks and/or tethering arms, it is also possible to attach REs with different molecular structures that bind to different receptors, onto the same article scaffold in well-controlled proportions. Reactions using orthogonal reactive pairs can be done simultaneously or sequentially.

The RE must contain a functionality that allows its attachment to the article. Preferentially, although not necessarily, this functionality is one member of a pair of chemoselective reagents selected to aid the coupling reaction (Lemieux, G., Bertozzi, C., *Trends in Biotechnology*, 1998, 16, 506-513). For example, when the article surface (and/or linkers grafted to its surface) displays a haloacetal, a peptide RE may be attached through a sulfhydryl moiety. A sulfhydryl moiety in the RE structure can be accomplished through inclusion of a cysteine residue.

Coupling is also possible between a primary amine on the article or the linker terminus and a carboxylic acid on the RE. A carboxylate in the peptide structure can be found either on its terminal amino acid, for linear peptides, or through the inclusion of aspartic or glutamic acid residues. The opposite configuration, where the carboxylic

acid is on the article and a primary amine belongs to the peptide, is also easily accessible. Many polymerizable building blocks contain acidic moieties, which are accessible at the surface of the beads after their polymerization. As for poly(amino acid)-based REs, a primary amine function can be found either at its N-terminus (if it is linear) and/or via introduction of a lysine residue.

Another example of reactive chemical pairs consists of the coupling of a sulfhydryl with a haloacetal or maleimide moiety. The maleimide function can be easily introduced, either on a peptide, a linker, or the surface of the articles, by reacting other common functionalities (such as carboxylic acids, amines, thiols or alcohols) with linkers through methods known to one of skill in the art, such as described for example by G. T. Hermanson in *Bioconjugate Techniques*, Academic Press Ed., 1996.

Peptides can also be coupled to the article and/or the tether with a reaction between an amino-oxy function and an aldehyde or ketone moiety. The amino-oxy moiety (either on the articles or in the peptide) can be introduced, starting from other common functionalities (such as amines for example), by a series of transformations known to those skilled in the art. In the same way, aldehyde- or ketone-containing articles and aldehyde-containing peptides are readily synthesized by known methods.

The resulting RE-functionalized, drug-containing articles may be used immediately, may be stored as a liquid solution, or may be lyophilized for long-term storage.

The REs may be any small or large molecular structure that provides the desired binding interaction(s) with the cell surface receptors of the targeted molecule. The number of recognition element moieties per article can range from 2 to about 1000, preferably from 2 to 500, and most preferably from 2 to 100. The articles may optionally further be comprised of more than one type of RE. As used herein, a RE "type" is defined as a specific molecular structure.

In one embodiment REs are comprised of peptides. Peptides used as REs according to this invention will generally possess dissociation constants between 10^{-4} and 10^{-9} M or lower. Such REs may be comprised of known peptide ligands. For instance, Phoenix Peptides' peptide ligand-receptor library (http://www.phoenixpeptide.com/Peptidelibrarylist.htm) contains thousands of known

peptide ligands to receptors of potential therapeutic value. The peptides may be natural peptides such as, for example, lactams, dalargin and other enkaphalins, endorphins, angiotensin II, gonadotropin releasing hormone, melanocyte-stimulating hormone, thrombin receptor fragment, myelin, and antigenic peptides. Peptide building blocks useful in this invention may be discovered via high throughput screening of peptide libraries (e.g. phage display libraries or libraries of linear sequences displayed on beads) to a protein of interest. Such screening methods are known in the art (for example, see C.F. Barbas, D. R. Burton, J. K. Scott, G. J. Silverman, *Phage Display*, 2001, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). The high affinity peptides may be comprised of naturally-occurring amino acids, modified amino acids or completely synthetic amino acids. The length of the recognition portion of the peptide can vary from about 3 to about 100 amino acids. Preferably, the recognition portion of the peptide ranges from about 3 to about 15 amino acids, and more preferably from 3 to 10 amino acids. Shorter sequences are preferred because peptides of less than 15 amino acids may be less immunogenic compared to longer peptide sequences. Small peptides have the additional advantage that their libraries can be rapidly screened. Also, they may be more easily synthesized using solid-state techniques.

Particular peptides of interest include those comprised of the amino acid sequence RGD that binds to Integrin receptors, NGR that binds to aminopeptidase-N, YCPIWKFPDEECY, or other sequences found in Greene, et.al., *J. Biol. Chem.*, 2002, 277(31), 28330-28339, that bind to erbB1; peptides comprised of the amino acid sequence CdFCDGFdYACYMDV, where dF and dY representing the D isomer of the amino acid residues or other sequences delineated in Murali, *J. Med. Chem.*, 2001, 44, 2565 - 2574, as REs; peptides disclosed in PCT WO 01/74849 that bind to CEA; and peptides comprised of the amino acid sequence ATWLPPR, as described in Demangel, et.al., *EMBO J.*, 2000, 19(7), 1525-1533.

REs may be comprised of a variety of other molecular structures, including vitamins such as folate, growth factors such as EGF, proteins such as transferrin, antibodies, antibody fragments, lectins, nucleic acids, and other receptor ligands. Humanized or fully human antibodies, and humanized or fully human antibody fragments are preferred for use in the present invention.

Additionally, it will be possible to design other non-protein compounds to be employed as the RE, using techniques known to those working in the area of drug design. Such methods include, but are not limited to, self-consistent field (SCF) analysis, configuration interaction (CI) analysis, and normal mode dynamics computer programs, all of which are well described in the scientific literature. See, Rein et al., *Computer-Assisted Modeling of Receptor-Ligand Interactions*, Alan Liss, New York (1989). Preparation of non-protein compounds and moieties will depend on their structure and other characteristics and may normally be achieved by standard chemical synthesis techniques. See, for example, *Methods in Carbohydrate Chemistry, Vols. I-VII*; Analysis and Preparation of Sugars, Whistler et al., Eds., Academic Press, Inc., Orlando (1962), the disclosures of which are incorporated herein by reference.

The use of multiple RE molecules of the same molecular structure or of different molecular structure to make up the article can increase the avidity of the article. As used in the present invention, "high affinity" means a binding of a single RE to a single target molecule with a binding constant stronger than 10^{-4} M, while "avidity" means the binding of two or more such RE units to two or more target molecules on a cell or molecular complex.

The number of REs per nanoarticle can range from 2 to about 1000, preferably from 2 to 500. The nanoarticles may optionally further be comprised of more than one type of RE. As used herein, a RE "type" is defined as an RE of a specific molecular structure. An additional advantage of the present invention is that multiple RE types with complementary features may be incorporated into a single nanoarticle.

Also disclosed herein is a method for releasing the bioactive molecules from the articles and for potentiating bioactivity. Under the influence of an alternating magnetic field, the temperature of the magnetic core material and thus of the entire article may be raised through various mechanisms described in the literature. This heating results in the disruption of the bonds holding the article of the instant invention together and/or the coordination bonds retaining the bioactive platinum agent within the complex, thus causing the accelerated release of the bioactive agent from the article. Preferably, some individual platinum atoms are each connected to two or more distinct polymer chains, which contributes to the stabilization of the nanoarticle network.

In the case of chemotherapeutics, elevated temperature is known to increase the toxicity and anti-cancer potency of chemotherapeutics, so the localized heating generated by the magnetic material is expected to increase the potency of the chemotherapeutic released from the article. While the heating itself is expected to result in the death of a portion of cancer cells in a tumor, heating of the entire tumor may not be achieved. However, the heating of the surrounding tissue is expected to aid in the diffusion of the chemotherapeutic into the tumor, resulting in the killing of more cancer cells. Outside of the applied magnetic field wherein the articles are heated, the articles of the invention will release the chemotherapeutic toxins only to a limited degree, such that the toxicity of the chemotherapeutic both systemically and in certain organs, such as in the heart, liver, kidney, and lung, is much reduced.

Thus, at least three targeting mechanisms localize articles of the instant invention at the tumor site. First, the article size will be designed such that preferential tumor accumulation occurs through the well-documented enhanced permeability and retention (EPR) effect. Second, targeting elements on the surface of the nanoarticles will help localize the particles to the tumor site by binding tumor-associated antigens. Several different targeting agents may be utilized, including specific small molecule or peptide ligands, as well as antibodies or antibody fragments (e.g., scFv). Finally, application of a localized alternating magnetic field (AMF) at the tumor site can be used to cause localized release of the chemotherapeutic. The AMF will also lead to heating within the cancer tissue, killing some tumor cells by this mechanism and, perhaps more importantly, increasing the vulnerability of the tumor cells to the released cytotoxic payload.

Advantageously, the location of the articles of the instant invention within the mammalian body may be determined using magnetic resonance imaging (MRI) of the superparamagnetic core material.

The invention is further directed to methods of synthesizing these nanoarticles. The manufacturing of the articles of the invention, specifically certain purification steps, may be facilitated by the articles' magnetic components.

While the articles of the invention may be larger in size, they are preferably from about 5 nm to about 1000 nm, more preferably from about 5 nm to about 500 nm, in

diameter. Because of their size and hydrogel structure, the nanoarticles may circulate in the blood stream without being eliminated by the kidney or taken up by the RE system, and may localize in pathological tissue via passage through the pathological tissue's leaky vasculature; the incorporation of targeting agents can further increase article accumulation in tissue to be treated, as described below.

Article Scaffold Fabrication in Reverse Microemulsions: Components of the present invention can be fabricated using of reverse microemulsions. In one embodiment, magnetic colloids are formed in the dispersed aqueous phase of a reverse microemulsion. Next, hydrogel scaffolds are formed around the magnetic core through the addition of hydrophilic building blocks to the reverse microemulsion containing the magnetic colloids. The hydrophilic building blocks are then polymerized, forming a magnetic colloid-containing hydrogel nanoarticle. The organic solvent and non-reactive surfactants are removed after polymerization to yield crosslinked, water-soluble, magnetite-cored nanoscopic articles. Chemotherapeutics may be incorporated either during or after hydrogel formation.

Reverse microemulsions for magnetic colloid and scaffold fabrication are formed by combining aqueous buffer or water, building blocks, organic solvent, surfactants and initiators in the appropriate ratios to yield a stable phase of surfactant-stabilized aqueous nanodroplets dispersed in a continuous oil phase. Stable reverse microemulsion formulations can be found using known methods by those skilled in the art. They are discussed, for example, in *Microemulsion Systems*, edited by H. L. Rosano and M. Clausse, New York, N.Y., M. Dekker, 1987; and in *Handbook of Microemulsion Science and Technology*, edited by P. Kumar and K.L. Mittel, New York, N.Y., M. Dekker, 1999. In this invention, an aqueous phase with solubilized hydrophilic building blocks is added to an organic solvent containing one or more solubilized surfactants to form a reverse microemulsion.

The dispersed aqueous phase contains hydrophilic building blocks solubilized at about 5 to about 65 wt%, preferably about 5 to about 25 wt%, most preferably 10 to 20 wt%. While not wishing to be bound by theory, the use of high water-content hydrogel scaffolds also may reduce immunogenicity in end uses, because there is less foreign surface for immune system components to recognize. The high water content also

provides compliancy through a more flexible scaffold. Thus, when attaching to cell surface receptors, the articles are able to conform to the cell surface, allowing more surface receptors to be bound. Binding more receptors may allow the article to better function as an antagonist. Additionally, while not wishing to be bound by theory, it is believed that article cell surface coverage can inhibit other cell signaling pathways.

Polymerization of the building blocks in the nanodroplets of the dispersed aqueous phase of the reverse microemulsion follows procedures known to those skilled in the art (see, for example, Odian G.G., *Principles of Polymerization, 3rd Ed.*, Wiley, New York, 1991; L.H. Sperling, *Introduction to Physical Polymer Science*, Chapter 1, pp. 1-21, John Wiley and Sons, New York, 1986; and R.B. Seymour and C.E. Carraher, *Polymer Chemistry*, Chapters 7-11, pp. 193-356, Dekker, New York, 1981). Polymerization has been performed in the dispersed phase of microemulsions and reverse microemulsions (for a review, see Antonietti, M.; and Basten, R., Macromol. *Chem. Phys.* 1995, 196, 441; for a study of the polymerization of a hydrophilic monomer in the dispersed aqueous phase of a reverse microemulsion, see Holtzscherer, C.; and Candau, F., *Colloids and Surfaces*, 1988, 29, 411). Such polymerization may yield articles in the 5 nm to 50 nm size range.

The size of the nanodroplets of the dispersed aqueous phase is determined by the relative amounts of water, surfactant and oil phases employed. Surfactants are utilized to stabilize the reverse microemulsion. These surfactants do not include crosslinkable moieties; they are not building blocks. Surfactants that may be used include commercially available surfactants such as Aerosol OT (AOT), polyethyleneoxy(n)nonylphenol (Igepal™, Rhodia Inc. Surfactants and Specialties, Cranbrook, NJ), sorbitan esters including sorbitan monooleate (Span® 80), sorbitan monolaurate (Span® 20), sorbitan monopalmitate (Span® 40), sorbitan monostearate (Span® 60), sorbitan trioleate (Span® 85), and sorbitan tristearate (Span® 65), which are available, for example, from Sigma (St Louis, MO). Sorbitan sesquioleate (Span® 83) is available from Aldrich Chemical Co., Inc. (Milwaukee, WI). Other surfactants that may be used include polyoxyethylenesorbitan (Tween®) compounds, including polyoxyethylenesorbitan monolaurate (Tween® 20 and Tween® 21), polyoxyethylenesorbitan monooleate (Tween® 80 and Tween® 80R),

polyoxyethylenesorbitan monopalmitate (Tween® 40), polyoxyethylenesorbitan monostearate (Tween® 60 and Tween® 61), polyoxyethylenesorbitan trioleate (Tween® 85), and polyoxyethylenesorbitan tristearate (Tween® 65), which are available, for example, from Sigma (St Louis, MO). Other exemplary commercially available surfactants include polyethyleneoxy(40)-sorbitol hexaoleate ester (Atlas G-1086, ICI Specialties, Wilmington DE), hexadecyltrimethylammonium bromide (CTAB, Aldrich), and linear alkylbenzene sulfonates (LAS, Ashland Chemical Co., Columbus, OH).

Other exemplary surfactants include fatty acid soaps, alkyl phosphates and dialkylphosphates, alkyl sulfates, alkyl sulfonates, primary amine salts, secondary amine salts, tertiary amine salts, quaternary amine salts, n-alkyl xanthates, n-alkyl ethoxylated sulfates, dialkyl sulfosuccinate salts, n-alkyl dimethyl betaines, n-alkyl phenyl polyoxyethylene ethers, n-alkyl polyoxyethylene ethers, sorbitan esters, polyethyleneoxy sorbitan esters, sorbitol esters and polyethyleneoxy sorbitol esters.

Other surfactants include lipids, such as phospholipids, glycolipids, cholesterol and cholesterol derivatives. Exemplary lipids include fatty acids or molecules comprising fatty acids, wherein the fatty acids include, for example, palmitate, oleate, laurate, myristate, stearate, arachidate, behenate, lignocerate, palmitoleate, linoleate, linolenate, and arachidonate, and salts thereof such as sodium salts. The fatty acids may be modified, for example, by conversion of the acid functionality to a sulfonate by a coupling reaction to a small molecule containing that moiety, or by other functional group conversions known to those skilled in the art.

Additionally, polyvinyl alcohol (PVA), polyvinylpirolidone (PVP), starch and their derivatives may find use as surfactants in the present invention.

Cationic lipids may be used as cosurfactants, such as cetyl trimethylammonium bromide/chloride (CTAB/CTAC), dioctadecyl dimethyl ammonium bromide/chloride (DODAB/DODAC), 1,2-diacyl-3-trimethylammonium propane (DOTAP), 1,2-diacyl-3-dimethyl ammonium propane (DODAP), [2,3-bis(oleoyl)propyl] trimethyl ammonium chloride (DOTMA), and [N-(N'-dimethylaminoethane)-carbamoyl]cholesterol, dioleoyl) (DC-Chol). Alcohols may also be used as cosurfactants, such as propanol, butanol,

pentanol, hexanol, heptanol and octanol. Other alcohols with longer carbon chains may also be used.

The articles of the invention can be administered by injection (subcutaneous, intravenous, intramuscular, intradermal, intraperitoneal, intracerebral, or parenteral), with intraveneous injection being a preferred route. The articles may also be suitable for nasal, pulmonary, vaginal, ocular delivery and oral administration. The articles may be suspended in a pharmaceutically acceptable carrier for administration.

Reagents and starting materials in some embodiments can be obtained commercially from chemical distributors such as Sigma-Aldrich (St Louise, MO and Milwaukee, WI), Kodak (Rochester, NY), Fisher (Pittsburgh, PA), Pierce Chemical Company (Rockford, IL), Carbomer Inc. (Westborough, MA). PEG compounds may be purchased through companies such as NOF America Corporation (White Plains, NY), and Nektar (Birmingham, AL). Peptides to be used as REs can be purchased from many sources, one being Bachem (King of Prussia, PA). Proteins may be obtained from sources such as Calbiochem (San Diego, CA).